

## SEQUENCE LISTING

<110> Cox III, George Norbert  
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 Sangamo Biosciences, Inc.

<120> Regulation of Endogenous Gene Expression in Cells Using  
 Zinc Finger Proteins

<130> 019496-002200US

<140> 09/229,037  
 <141> 1999-01-12

<160> 40

<170> PatentIn Ver. 2.0

<210> 1  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:exemplary motif  
 of C2H2 class of zinc finger proteins (ZFP)

<220>  
 <221> MOD\_RES  
 <222> (2)..(3)  
 <223> Xaa = any amino acid

<220>  
 <221> MOD\_RES  
 <222> (4)..(5)  
 <223> Xaa = any amino acid, may be present or absent

<220>  
 <221> MOD\_RES  
 <222> (7)..(18)  
 <223> Xaa = any amino acid

<220>  
 <221> MOD\_RES  
 <222> (20)..(22)  
 <223> Xaa = any amino acid

<220>  
 <221> MOD\_RES  
 <222> (23)..(24)  
 <223> Xaa = any amino acid, may be present or absent

<400> 1  
 Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 1 5 10 15  
 Xaa Xaa His Xaa Xaa Xaa Xaa Xaa His  
 20 25

<210> 2  
 <211> 10  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:ZFP target site  
 with two overlapping D-able subsites

<220>  
 <221> modified\_base  
 <222> (1)..(2)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (5)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (8)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (9)  
 <223> n = a,c or t; if g, then position 10 cannot be g  
 or t

<220>  
 <221> modified\_base  
 <222> (10)  
 <223> n = a or c; if g or t, then position 9 cannot be g

<400> 2  
 nngkngknnn

10

<210> 3  
 <211> 10  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:ZFP target site  
 with three overlapping D-able subsites

<220>  
 <221> modified\_base  
 <222> (1)..(2)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (5)  
 <223> n = g,a,c or t

<220>  
 <221> modified\_base  
 <222> (8)  
 <223> n = g,a,c or t

<400> 3  
 nngkngkngk

10

<210> 4  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 4  
 Asp Gly Gly Gly Ser  
     1                    5

<210> 5  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 5  
 Thr Gly Glu Lys Pro  
     1                    5

<210> 6  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 6  
 Leu Arg Gln Lys Asp Gly Glu Arg Pro  
     1                    5

<210> 7  
 <211> 4  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:linker

<400> 7  
 Gly Gly Arg Arg  
     1

<220>  
<223> Description of Artificial Sequence:linker

&lt;400&gt; 12

Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Gly Ser Glu Arg Pro  
 1 5 10 15

&lt;210&gt; 13

&lt;211&gt; 25

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:ZFP target site  
 region surrounding initiation site of vascular  
 endothelial growth factor (VEGF) gene containing  
 two 9-base pair target sites

&lt;220&gt;

&lt;221&gt; protein\_bind

&lt;222&gt; (4)..(12)

&lt;223&gt; upstream 9-base pair ZFP VEGF1 target site

&lt;220&gt;

&lt;221&gt; protein\_bind

&lt;222&gt; (14)..(22)

&lt;223&gt; downstream 9-base pair ZFP VEGF3a target site

&lt;400&gt; 13

agcggggagg atcgcgagg cttgg

25

&lt;210&gt; 14

&lt;211&gt; 298

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:VEGF1 ZFP  
 construct targeting upstream 9-base pair target  
 site in VEGF promoter

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (2)..(298)

&lt;223&gt; VEGF1

&lt;400&gt; 14

g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg cgc 97  
 Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg  
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145  
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
 35 40 45

aaa cgc ttc acc cgt tgc tca aac ctg cag cgt cac aag cgt acc cac 193  
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
 50 55 60

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241  
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag aag 289  
 Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

ggt gga tcc 298  
 Gly Gly Ser

<210> 15  
 <211> 99  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF1 ZFP  
 construct targeting upstream 9-base pair target  
 site in VEGF promoter

<400> 15  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg  
 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

Gly Gly Ser

<210> 16  
 <211> 298  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF3a ZFP  
 construct targeting downstream 9-base pair target  
 site in VEGF promoter

<220>  
 <221> CDS  
 <222> (2)..(298)  
 <223> VEGF3a

&lt;400&gt; 16

g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97  
 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145  
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
 35 40 45

aaa cgc ttc acc cgt tcg tca aac cta cag agg cac aag cgt aca cac 193  
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
 50 55 60

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241  
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

cga agt gac gag ctg tca cga cat atc aag acc cac cag aac aag aag 289  
 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

ggt gga tcc 298  
 Gly Gly Ser

&lt;210&gt; 17

&lt;211&gt; 99

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: VEGF3a ZFP  
 construct targeting downstream 9-base pair target  
 site in VEGF promoter

&lt;400&gt; 17

Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
 1 5 10 15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
 65 70 75 80

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
 85 90 95

Gly Gly Ser

29

<210> 22  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer SPE7

<400> 22  
 gagcagaatt cggcaagaag aagcagcac 29

<210> 23  
 <211> 26  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer SPEamp12

<400> 23  
 gtggtctaga cagctcgtca cttcgc 26

<210> 24  
 <211> 28  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer SPE  
 ampl3

<400> 24  
 ggagccaagg ctgtggtaaa gtttacgg 28

<210> 25  
 <211> 26  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:primer SPEamp11

<400> 25  
 ggagaagctt ggatcctcat tatccc 26

<210> 26  
 <211> 83  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:sequence  
 ligated between XbaI and StyI sites

&lt;400&gt; 26

tctagacaca tcaaaaccca ccagaacaag aaagacggcg gtggcagcgg caaaaagaaa 60  
 cagcacatat gtcacatcca agg 83

&lt;210&gt; 27

&lt;211&gt; 39

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:primer GB19

&lt;400&gt; 27

gccatgccgg tacccatacc tggcaagaag aagcagcac 39

&lt;210&gt; 28

&lt;211&gt; 33

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:primer GB10

&lt;400&gt; 28

cagatcggat ccacccttct tattctggtg ggt 33

&lt;210&gt; 29

&lt;211&gt; 589

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:designed  
 6-finger ZFP VEGF3a/1 from KpnI to BamHI

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (2)..(589)

&lt;223&gt; VEGF3a/1

&lt;400&gt; 29

g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
     1                    5                    10                    15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97  
 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
                     20                    25                    30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145  
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
                     35                    40                    45

aaa cgc ttc aca cgt tcg tca aac cta cag agg cac aag cgt aca cac 193  
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
                     50                    55                    60

```
<210> 30
<211> 196
<212> PRT
<213> Artificial Sequence
```

```
<400> 30  
Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
1          5              10                15  
  
Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
          20              25              30  
  
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
        35              40              45  
  
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
      50              55              60  
  
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
    65              70              75              80
```

25

```
<400> 37
ggtacccggg gatcccgac actggtgacc ttcaaggatg tatttgtgga cttcaccagg 60
gaggagtgga agctgctgga cactgctcag cagatcgtgt acagaaatgt gatgctggag 120
```

```
<210> 38
<211> 277
<212> DNA
<213> Artificial Sequence
```

```
<400> 38
ggatccgccc ccccgaccga tgtcagcctg ggggacgagc tccacttaga cggcgaggac 60
gtggcgatgg cgcattgccga cgcgctagac gatttcgata tggacatggt gggggacggg 120
gattccccgg ggccggggatt tccccccac gactccgccc cctacggcgc tctggatatg 180
gccgacttcg agtttgagca gatgtttacc gatgcccttg gaattgacga gtacggtggg 240
ggcagcgact acaaggacga cgatgacaag taagctt 277
```

```
<220>
<223> Description of Artificial Sequence:sequence
      replacing NLS-KRAB-FLAG with NLS-FLAG only
```

```
<400> 39
gaattcgcta gcgccaccat ggcccccaag aagaagagga aggtgggaat ccatggggta 60
cccgggggatg gatccggcag cgactacaag gacgacgatg acaagtaagc ttctcgag 118
```

```
<210> 40
<211> 204
<212> DNA
<213> Artificial Sequence
```

<220>  
<223> Description of Artificial Sequence:insert into  
MluI/BglII sites of pGL3-Control to create  
pVFR1-4x

```
<400> 40
acgcgtaagc ttgctagcga gcggggagga tcgcggaggc ttggggcgagc cgggtagagc 60
gagcggggag gatcgcgagg gccttggggca gccgggtaga gcgagcgggg aggatcgcgg 120
aggcttgggg cagccgggta gagcgagcgg ggaggatcgc ggaggcttgg ggcagccggg 180
tagagcgctc agaagcttag atct                                     204
```